Annu. Rev. Med. 2002. 53:207–21

CLINICAL TRIALS OF HIV VACCINES*

Barney S. Graham
Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 40 Convent Drive, Building 40, Room 2502, MSC-3017, Bethesda, Maryland 20892-3017; e-mail: bgraham@nih.gov

Key Words AIDS, virus, immunity, neutralizing antibody, cytotoxic T lymphocyte

■ **Abstract** Development of a preventive vaccine for HIV is the best hope of controlling the AIDS pandemic. Evidence from natural history studies and experiments in animal models indicates that immunity against HIV is possible, suggesting that vaccine development is feasible. These studies have shown that sufficient levels of neutralizing antibody against HIV can prevent infection, although the effect is type-specific. In contrast, HIV-specific cytotoxic T lymphocyte (CTL) activity has broad cross-reactivity, and although CTL activity alone cannot prevent HIV infection, it can control the level of viremia at a low level. Evaluation of candidate vaccines in human trials has focused on approaches that can safely elicit HIV-specific antibody and T cell responses. Current strategies have been unable to induce antibody with broad neutralizing activity against primary HIV isolates. However, recombinant poxvirus and DNA vaccines have elicited CTL responses that are broadly cross-reactive against primary HIV isolates from diverse clades. Future advances will require the discovery of new immunogens that can induce neutralizing antibody, as well as efficacy trial evaluation of regimens optimized for CTL induction.

INTRODUCTION

The HIV-1 pandemic has become one of the greatest infectious disease threats to human health and social stability that the world has ever encountered. Nearly 40 million persons are living with HIV-1 infection and more than 21 million have already died from HIV-induced disease. Although effective antiretroviral therapy has slowed the epidemic in some industrialized countries, worldwide there are still an estimated 15,000 new HIV infections occurring daily. In addition to vast personal suffering and the loss of young adult parents, caretakers, and wage-earners, HIV has created an unprecedented strain on the social and economic infrastructure of many developing countries, particularly in sub-Saharan Africa. These facts make it imperative that the epidemic be controlled as rapidly as possible

through prevention of new infections. Although education and available public health approaches should be vigorously pursued, development of a preventive vaccine is the best hope of controlling the HIV epidemic.

New molecular tools in virology and immunology, new adjuvants, new gene expression systems, new antigen delivery systems, recent discoveries concerning HIV entry and pathogenesis, evidence that natural immunity is achieved in rare instances, and promising studies of candidate vaccines in animal models have provided reasons to hope for a safe and effective AIDS vaccine. However, some have argued that preventive vaccination for AIDS will not be possible (1), and the complex biology of HIV-1 makes its development a daunting task.

EXPECTATIONS OF A VACCINE AGAINST HIV

Vaccines developed during the last century have provided unprecedented health and freedom from epidemics of many previously common infectious diseases. They have worked by protecting the vaccinated individual from the consequences of infection, but also by reducing the incidence of transmission within the population, diminishing the spread of epidemics. The ultimate goal for HIV vaccine development is to prevent infection in an exposed individual or induce rapid clearance of infected cells to avoid persistent infection. However, no current licensed vaccine for other viral pathogens is known to fully prevent infection, and most are effective because they limit the replication and spread of the pathogen below the threshold for clinical expression of disease. It is unlikely that vaccine-induced immune responses will be able to prevent the establishment of latency. It has been shown that a small proportion of infected CD4+ T cells become quiescent, allowing viral latency to be established in a reservoir of long-lived cells (1a, this volume). Therefore, a more realistic initial goal for HIV vaccine development is to dampen the initial viremia in an infected individual, maintain a low virus load, and prevent progression to AIDS.

Altering the disease course in individuals could potentially have a large impact on the spread of HIV within a population. The determinants of epidemic spread can be expressed as $R_o = \beta \times c \times D$, where R_o is the reproductive rate of the epidemic or a measure of spread; β is the transmission efficiency of the agent; c is the frequency of new partners or new transmission opportunities; and D is the duration of transmissibility. If $R_o > 1$, the epidemic will spread, and if $R_o < 1$ the epidemic will diminish (2). Effective vaccination has the potential to change both β and D, while education, surveillance, and traditional public health approaches can alter c. The significant impact on the incidence of new infections from the use of highly active antiretroviral treatment (HAART), as well as natural history studies, encourage the idea that vaccine-induced immune responses that maintain a low viral load in infected individuals will reduce transmission efficiency in the population (3). Therefore, the initial aim in vaccine development is to identify approaches that will induce immune responses that control infection and prevent disease in individuals, and slow the epidemic spread of HIV within a population.

ASSUMPTIONS RELEVANT TO HIV VACCINE DESIGN

Vaccine-Induced Immunity is Possible

Although many challenges remain, three observations suggest that vaccine development is feasible. First, HIV transmission is relatively inefficient. On average, more than 200 exposures are required to cause one infection in settings of sexual transmission (4) or needle-stick injuries (5). Therefore, modest improvement in antiviral defenses may have a profound impact on the transmissibility of HIV. Furthermore, based on analysis of HIV isolates in acute infection, most individuals are infected with a very small number of infectious particles, in many cases a single virion (6, 7). The small inoculum size improves the chances that vaccine-induced immunity could prevent infection. In addition, these data suggest that transmitted virus may have limited structural and genotypic features, which would further improve the chances for identifying mechanisms of protective immunity. Second, there are examples of natural immunity from studies of highly exposed, uninfected (8, 9), and long-term nonprogressor (10) populations. There is also evidence from western Africa, where there are concurrent epidemics of HIV-1 and HIV-2, that prior infection with the less virulent HIV-2 confers some protection against HIV-1 infection (11). Finally, there are now many examples of passive protection and vaccine-induced immunity in nonhuman primate models of lentivirus infection.

Timing of the HIV-Specific Immune Response is Critical

Several parameters of the vaccine-induced immune response determine its ability to protect the host from infection or disease, including specificity, functional properties, magnitude, and compartmentalization. Another critical factor is timing. The timing of the immune response with respect to initial virus infection and spread is particularly important in the case of HIV-1 infection. One reason is that the longer HIV-1 replicates in the host, the more diverse variants evolve, which may allow escape from subsequent immune responses. In addition, once HIV-1 resides in the extracellular space of lymph node germinal centers and in latently infected cellular reservoirs, or is sequestered in the central nervous system and other sites that are relatively protected from immune responses, it probably cannot be fully eliminated from the host.

After the initial burst of virus replication and high-titer viremia, the titer of virus in plasma is reduced by the initial immune responses and establishes a new plateau about 6 months after infection, referred to as the viral load "set point." The immune response to HIV-1 infection includes potent effector responses that at best achieve a steady state in which virus clearance matches virus production (11a, this volume). The magnitude of the viral load "set point" correlates with the rate of immune system destruction (12). The important advantage of vaccine-induced immune responses is that they are induced prior to infection and can be recalled more rapidly than primary effector mechanisms. Therefore, the success

of vaccination may hinge on altering events that occur in the early hours following HIV-1 exposure.

Vaccines Work By Inducing Adaptive Immune Responses

Preventive vaccines work by establishing immunologic memory for antigenic structures presented by the pathogen or by infected cells. Therefore, the immunologic "tool box" accessible for vaccine-induced immunity only includes elements of the adaptive immune response. The basic cellular elements of adaptive immunity include the B and T lymphocytes. The primary effector mechanisms important for protection against viruses are antibodies produced by B cells and cytotoxic activity mediated primarily by CD8+ T cells. In addition, soluble factors produced by activated CD4+ and CD8+ T cells have antiviral activity and can influence the differentiation, expansion, and duration of T cell responses. Elements of the nonadaptive immune system are important during the initial phases of antigen presentation and development of the cytokine microenvironment, mediating many of the activities induced by adjuvants. However, immunity against subsequent infection is determined by adaptive immune responses with memory for key antigens and functional effector activities that can neutralize the pathogen and rapidly eliminate infected cells.

Neutralizing Antibody and Cytotoxic T Cells are the Major Effectors of Antiviral Immunity

The correlates of immunity against HIV-1 have not been defined in an absolute sense, but much is known about HIV-specific immune responses associated with long-term survival and maintenance of low viral loads (11a, this volume). In addition, there is a general understanding about how different elements of the adaptive immune response should work, and these concepts can be tested against observations made in studies of the natural history of HIV infection in humans or experimental data from animal models (13). Alternative vaccine-inducible effector mechanisms mediated by chemokines and other soluble factors produced by T cells may ultimately be shown to have a role in protection (14, 15), but in this review I focus on classical neutralizing antibody and CD8+ cytotoxic T cell activities.

There is debate and speculation about which component of the adaptive immune system is most important for immunity. However, abundant evidence indicates that in HIV and other virus infections, both antibody and CD8+ CTL are important and perform complementary roles in protection from and control of infection. CD4+ T cells are also of obvious importance, especially for influencing differentiation patterns and expansion of selected lymphocyte populations, but their role as a direct effector of virus clearance is less clear. Therefore, another assumption is that CD4+ T cells will be induced in the process of achieving the appropriate antibody and CD8+ CTL responses. CD4+ T cells are not specifically addressed in this paper.

Antibody is the only component of the adaptive immune response that can neutralize a virus particle prior to infection of a cell and is the only immune response associated with protection for any currently licensed vaccines. Antibody titers can be sustained at high levels in serum and in mucosal secretions and be present at the time of infection. T cells, in contrast, recognize virus only in the context of an already infected cell and require a few days for activation and expansion of memory populations to respond. Therefore, an effective neutralizing antibody response is likely to be a critical component of vaccine-induced immunity, because it can prevent infection and thereby reduce inoculum size and establishment of latently infected cells.

Neutralization is defined as the ability to reduce infectivity of cell-free virus, usually measured in susceptible cells in culture. Although this aspect of antibody activity is thought to be the key function associated with protection from infection, there is some debate about its mechanism. The identification of specific neutralizing epitopes suggests that the site of antibody binding is important. However, it has also been suggested that neutralization occurs when a threshold level of the virion surface is covered by antibody that binds the native envelope oligomer regardless of specificity (16). In either case, it is clear that T cell line–adapted viruses are more susceptible to neutralization than primary field isolates, which presents a major obstacle to achieving this immunologic endpoint (17, 18).

T cells recognize virus-infected cells by specific interactions between the T cell receptor and 8–10 amino acid peptides processed from viral antigens and presented in the context of major histocompatibility complex (MHC) molecules. Therefore, T cells can only recognize and clear virus after infection has occurred. The recognition is restricted by the MHC molecule, which means that the particular epitopes recognized by a given individual depend on the set of inherited alleles encoding the MHC molecules. Although each person should have the capacity to recognize multiple epitopes among the antigens included in HIV-1, the hierarchy of recognition or epitope dominance may vary even among individuals who share MHC haplotypes. These issues suggest that the epitope repertoire in an HIV-1 vaccine will need enough breadth to encompass all the relevant MHC haplotypes of potential vaccinees. In addition, it will be important to induce a broad response in each individual against several viral antigens to diminish the possibility of immune escape through genetic variation and to allow for host selection of dominant epitopes.

The need for CD4+ T lymphocytes to initiate the adaptive immune response presents a dilemma, since these cells are the major targets for HIV-1 infection. The challenge is to effectively induce protective immunity against HIV-1 without risking infection of vaccine-induced HIV-specific CD4+ T cells. This emphasizes the need for effective immune responses, preexistent at the time of HIV exposure, so that virus clearance can be accomplished before the burden of infected cells is sufficient to maintain persistent infection. Although CD4+ T cells may have some capacity for lysis of HIV-infected cells (19) and production of antiviral cytokines, their major role is in shaping the immune response by establishing a

microenvironment with a particular cytokine composition. For HIV and most other viruses, induction of type 1 cytokines [production of interleukin(IL)-12, IL-2, and interferon (IFN)-γ] is more likely to provide protection than induction of type 2 cytokines (IL-4, IL-5, IL-13). Initial priming with vectors and the use of adjuvants other than alum (which promotes type 2 responses) would provide an advantage.

CD8+ T cells are the principal effector mechanism of the adaptive immune response to clear virus-infected cells. The CD8+ lymphocyte recognizes a virus-infected cell through a cognate interaction between the T cell receptor and a processed peptide epitope presented in the groove of an MHC class I molecule. The lysis of the infected cells occurs through the production and secretion of perforin and granzymes that penetrate the target cell membrane and induce apoptosis. FasL is also upregulated on the activated CD8+ T cell and can bind Fas on the target cell, providing another avenue for inducing apoptosis of the infected target cell. CD8+ T cells also produce cytokines with antiviral properties, such as IFN-γ and tumor necrosis factor (TNF)-α, in addition to other soluble factors that may play a role in virus inhibition. The T cell response causes cytopathology not only of the virus-infected cell but also to some degree in bystander cells. This again points to the importance of clearing virus rapidly to diminish the overall cytopathology and illness associated with the immune response to infection. For a more detailed review of immune control of HIV infection and HIV escape from immunity, see Chapter 9 (11a) and Chapter 28 (19a) in this volume.

DATA FROM STUDIES IN HUMANS AND ANIMALS

Antibody Can Prevent HIV Infection

Passive antibody studies in nonhuman primate models of lentivirus infection have directly proven that sufficient levels of neutralizing antibody can prevent infection. Studies evaluating polyclonal anti-HIV-1 antiserum (20) or monoclonal anti-V3 antibody in HIV-1 infected chimpanzees (20a) or polyclonal serum in SIV-infected macaques (21) have shown that when sufficiently high antibody titers are present prior to intravenous challenge, lentivirus infection can be prevented. Importantly, antibody-mediated protection has also been demonstrated against SHIV (a chimeric virus composed of an HIV envelope and SIV nucleocapsid and replication machinery) with an envelope glycoprotein derived from a dual tropic primary HIV isolate, and the protection could be correlated with in vitro neutralizing activity (22). More recently, passive prophylaxis using HIV immune globulin combined with two monoclonal antibodies has protected macaques from vaginal challenge with SHIV (23), and a mixture of three neutralizing monoclonal IgG1 antibodies given to pregnant macaques has protected their infants from SHIV oral challenge (24).

Definitive evidence of antibody-mediated protection in studies of active immunization has been more difficult to demonstrate, but an example from early studies performed with whole inactivated SIV vaccines is provocative. These studies

showed that antibodies to cell constituents incorporated into virions during production of challenge stocks were the best correlate of protection. When the virus used to produce vaccine was grown in human cells, and the virus challenge stock was grown in the same human cells, allogenic responses to the human proteins incorporated by the virus were the dominant mechanism of protection (25–28). Studies done with vaccine produced in monkey cells did not show consistent protection. Even though the antibody response was not specific for virus-encoded antigens, this example of vaccine-induced antibody-mediated protection suggests that protection through induction of virus-specific antibodies may be achievable. When SIV immune globulin was given one day after intravenous challenge with SIV, infection was not prevented, but disease progression was delayed in some animals (29). This again illustrates that the timing of immune responses is critical to the outcome of infection and that preexisting immunity gives the host a distinct advantage.

T Cells Can Control HIV Infection

Control of the initial viremia associated with primary HIV infection temporally correlates with the appearance of CD8+ cytotoxic T lymphocytes (30, 31), and mutations in specific CTL epitopes can be detected in the residual virus population (reviewed in 32). In addition, HIV-specific CD8+ CTL activity has been demonstrated in a small subset of uninfected, seronegative commercial sex workers in the Gambia and in Kenya, which suggests that transient infection may have occurred, inducing protective immunity mediated by CD8+ CTL (8, 9). In persons who remain uninfected despite significant occupational exposure to HIV-1–contaminated material, studies have also focused on HIV-specific T cell responses. Although HIV-specific antibodies cannot be detected, peripheral blood mononuclear cells show lymphoproliferative activity when stimulated with HIV-specific peptides (33). HIV-specific CTL responses have also been seen in this cohort (34), suggesting that transient infection may have occurred and been cleared by natural immune defenses.

Another subset of persons infected with HIV-1 have persistent infection but do not progress to AIDS for >12 years. Some of these individuals are infected with virus isolates that replicate poorly (35, 36). Others, though infected with viruses of normal replication capacity, have maintained a strong and broad set of humoral (36a) and cellular (37, 38) HIV-specific immune responses that may be responsible for their delayed disease progression. This phenomenon has been best associated with HIV-specific CD4+ T cell proliferation (37) and strong CD8+ CTL activity against multiple epitopes (38).

Another clue to the importance of T cell responses in the control of HIV has come from the evaluation of HIV-infected persons treated with HAART soon after primary infection. When these persons undergo structured treatment interruptions, there is a transient rise in the virus load, which results in a boost of functional T cell activity and subsequent control of virus load without HAART (39).

The most compelling evidence for the importance of CD8+ CTL for controlling lentivirus infection comes from studies of pathogenesis and vaccine evaluation in nonhuman primates. The CD8+ CTL response is the best correlate of viremia control after primary SIV infection in macaques (40). Several studies using nucleic acid or other recombinant vector approaches have demonstrated that induction of CD8+ CTL responses with a weak or absent antibody response does not prevent lentivirus infection but reduces viral load and delays disease progression. One of the early demonstrations of this was in macaques immunized with recombinant MVA (modified vaccinia Ankara) prior to challenge with SIV. Vaccination did not prevent infection, and the CTL cell response was associated with delayed disease progression (41). Subsequent studies have shown similar patterns (42–50). As attempts are made to optimize the CD8+ CTL response, such as the addition of an IL-2 adjuvant to a recombinant DNA vaccine regimen (49) or combining modalities of DNA and MVA (50), subsequent SHIV infection can be almost completely controlled. These data are consistent with the premise that vaccines able to establish a preexisting expanded population of HIV-specific CD8+ CTL are likely to delay disease progression in HIV-infected persons.

CLINICAL TRIALS OF CANDIDATE HIV VACCINES

Overview of Concepts Evaluated

Clinical trials have been performed in nearly 10,000 seronegative volunteers to evaluate the safety and immunogenicity of candidate AIDS vaccines. Recombinant envelope products, rgp120 or rgp160, produced in insect, yeast, or mammalian cells formulated with a variety of adjuvants, have been evaluated in clinical trials. Peptides tested to date have been derived from envelope V3 loop or gag sequences of clade B or multiple clades. They have been presented conjugated to an oligolysine backbone, as a lipopeptide conjugate, mixed with adjuvant, or as a fusion protein with the self-assembling yeast protein, Ty, as a particle. They have been administered intramuscularly in the deltoid or anterior thigh (to target lymph nodes that also drain the rectal mucosa), rectally and orally as Ty-gag virus-like particles, and orally encapsulated in polylactide copolymers. Live recombinant vectors including vaccinia, canarypox, and salmonella have been evaluated as well as nucleic acid–based vaccines. These vectors have been delivered by various routes and have been constructed to express either single or multiple HIV-1 antigens from both structural and nonstructural proteins.

New trials utilizing recombinant replication-incompetent adenovirus and MVA are just under way, as is a novel approach in which tat is the vaccine antigen. Tat is secreted from HIV-infected cells and has adverse effects on neighboring cells. It is hypothesized that blocking these effects with vaccine-induced antibody will facilitate virus clearance (51). These and other studies evaluating

schedule-of-administration and combination approaches using more than one product in the immunization regimen (52–54) are compiled and updated on the web site of the National Institutes of Health Vaccine Research Center (54). This table also documents studies that have advanced to Phase II and III status, and those that have been performed at sites outside the US. Fortunately, there have been no significant safety concerns other than unacceptable local reactogenicity associated with a few selected adjuvants (55).

Vaccine-Induced Antibody Responses in Clinical Trials

Neutralizing antibody responses have been induced by immunization with recombinant envelope glycoproteins alone or in combination with poxvirus vectors. The antibody response to immunization with rgp120 alone is in general maximal after the third or fourth injection, is dose-dependent, and can be attenuated unless there is a several-month interval between injections. Serum antibody titers have a relatively short half-life, and although they can be boosted, the titers generally achieve their peak level after the third or fourth injections. Repeated boosting does not prolong the half-life significantly. Therefore, it is likely that recombinant envelope glycoprotein products may find their greatest utility in boosting antibody responses in subjects primed with recombinant vector vaccines (56), or other strategies that can induce MHC class I–restricted CTL responses. This combination approach not only adds the CD8+ CTL component to the immune response but also results in a more durable antibody response.

The initial recombinant envelope glycoprotein products were derived from sequences of syncytium-inducing, T cell line–adapted (TCLA), CXCR-4–utilizing X4 viruses from clade B. Newer products, such as the VaxGen B/B product, incorporate sequences from primary isolates that utilize CCR5 (R5), combining the rgp120 from HIV-1_{MN} and the rgp120 from HIV-1_{GNE8} (57). Phase I and II studies have defined how dose, schedule, and formulation affect immunogenicity of purified protein subunit preparations as primary immunogens and as booster immunogens given in combination with other vaccine modalities. The principal findings related to vaccine-induced antibody responses in clinical trials of candidate HIV vaccines are that neutralizing antibody responses against TCLA viruses induced by the most immunogenic formulations are still five- to ten-fold lower than those produced by HIV-1 infection. The responses are type-specific with a relatively short half-life and are unable to neutralize typical primary isolate R5 viruses (54).

Vaccine-Induced CD8+ CTL Responses in Clinical Trials

Induction of HIV-specific CD8+ CTL responses generally requires the delivery of vaccine antigens into the cytoplasmic compartment of an antigen-presenting cell (APC) for display in an MHC class I molecule on the cell surface. Therefore, vector-based approaches or nucleic acid vaccines that rely on antigen production within the target cell are most effective. Delivering vaccine antigens as

purified proteins or even whole inactivated virus will primarily access the endocytic pathway for antigen presentation and lead to CD4+ T cell activation. Although this is critical for antibody production and important for supporting CD8+ CTL development, it is not sufficient for inducing CD8+ CTL. In some cases, a novel adjuvant or delivery system is able to provide access for these types of vaccines into the cytoplasmic compartment, but in general vector-based vaccines, including nucleic acids, are more potent methods for inducing CD8+ CTL. One exception is the use of peptides that incorporate a T cell epitope that can bind directly to an MHC class I molecule on the cell surface and induce CD8+ CTL responses.

Vector-based vaccines, beginning with recombinant vaccinia products, were first evaluated in clinical trials in the late 1980s with the expressed purpose of achieving vaccine-induced CD8+ CTL responses. The induction of CD8+ CTL responses has been a primary focus of clinical trials since the mid-1990s. It has been found that recombinant vaccinia expressing envelope glycoprotein only, or multiple antigens, can consistently induce long-lived CD8+ CTL responses in vaccinia-naive subjects (58–61). HIV-specific CD8+ CTL can also be detected in a majority of subjects receiving recombinant canarypoxvirus vectors, and in a subset, CTL activity is detectable for >18 months. The activity is at the threshold of detection in classical ^{51}Cr release assays requiring in vitro stimulation and is detected in only 15%–30% of subjects at any given time (62–66). However, unlike antibody responses, vaccine-induced CTL responses are broadly cross-reactive (67). CTLs induced by recombinant canarypox vectors have been shown to lyse target cells infected with primary R5 HIV-1 isolates from multiple clades (67). CD8+ CTL effectors have also been isolated from rectal mucosa from vaccinees, which suggests that T cells induced by parenteral vaccination may provide some level of protection at mucosal surfaces (M.J. McElrath et al., unpublished observations). Not only is classical MHC class I–restricted cytotoxic activity induced, but vaccine-induced noncytotoxic CD8+-mediated suppression of HIV-1 replication has also been demonstrated in recipients of recombinant canarypox vaccines (68). In summary, vaccine approaches that are currently being evaluated in clinical trials can induce HIV-specific CD8+ CTL activity that is durable and can lyse cells infected with typical primary R5 HIV-1 isolates from multiple clades. A phase III study evaluating the efficacy of combined recombinant canarypox with rgp120 is planned for Thailand, and a decision will be made in 2002 whether to advance this combination to phase III evaluation in the United States.

Recent advances in methods to quantitate T cells and evaluate their function are changing the process of vaccine evaluation. Enumeration of functional T cells by IFN-γ ELIspot or intracellular IFN-γ by FACS analysis, combined with identification of epitope-specific T cells with MHC-peptide tetramers by FACS analysis (69), has improved the ability to detect vaccine-induced responses by improving sensitivity and reproducibility. This allows the use of cryopreserved cells and reduces the number of effector cells needed.

FUTURE SCIENTIFIC CHALLENGES FOR HIV VACCINE DEVELOPMENT

Despite the current optimism, there are still many scientific obstacles to overcome in the development of a vaccine for HIV. Most important is the inability to induce broadly cross-reactive neutralizing antibody against typical primary HIV-1 isolates. This is one of several immune evasion strategies employed by HIV (19a, this volume). Without a high level of neutralizing activity present at the time of infection, it is unlikely that a vaccine-induced immune response can prevent the establishment of latency and infection of immunoprivileged sites. There are also questions involving the importance of HIV genetic variation, mucosal immunity, and duration of vaccine-induced immune responses that will be difficult to address until large-scale efficacy trials are implemented. In addition, many ethical, logistic, and economic challenges lie ahead.

In summary, the ultimate vaccine that can prevent persistent HIV-1 infection will probably require a conceptual breakthrough in the understanding of how to elicit broadly neutralizing antibody against primary R5 HIV-1 isolates. Development of such a vaccine will also involve a number of iterative steps to achieve optimal HIV-specific CD8+ CTL responses. However, a vaccine aimed at controlling viremia, delaying disease progression, and reducing transmission, based on induction of HIV-specific CD8+ CTL, could have a significant impact on the AIDS epidemic and may be within our grasp using currently available technology.

ACKNOWLEDGMENT

I thank R. Stokes Peebles for reviewing the manuscript.

LITERATURE CITED

1. Sabin AB. 1992. Improbability of effective vaccination against human immunodeficiency virus because of its intracellular transmission and rectal portal of entry. *Proc. Natl. Acad. Sci. USA* 89:8852–55

1a. Blankson JN, Persaud D, Siliciano RF. The challenge of viral reservoirs in HIV-1 infection. *Annu. Rev. Med.* 53:557–93

2. Holmes KK. 1994. Human ecology and behavior and sexually transmitted bacterial infections. *Proc. Natl. Acad. Sci. USA* 91:2448–55

3. Quinn TC, Wawer MJ, Sewankambo N, et al. 2001. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N. Engl. J. Med.* 342:921–29

4. Gray RH, Wawer MJ, Brookmeyer R, et al. 2001. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 357:1149–53

5. Geberding JL. 1994. Incidence and prevalence of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and cytomegalovirus among health care personnel at risk for blood exposure: final report

from a longitudinal study. *J. Infect. Dis.* 170:1410–17
6. Wolinsky SM, Wike CM, Korber BTM, et al. 1992. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. *Science* 255:1134–37
7. Wolfs TF, Zwart G, Bakker M, Goudsmit J. 1992. HIV-1 genomic RNA diversification following sexual and parenteral virus transmission. *Virology* 189:103–10
8. Fowke KR, Nagelkerke NJ, Kimani J, et al. 1996. Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. *Lancet* 348: 1347–51
9. Rowland-Jones S, Sutton J, Ariyoshi K, et al. 1995. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat. Med.* 1:59–64
10. Wagner R, Leschonsky B, Harrer E, et al. 1999. Molecular and functional analysis of a conserved CTL epitope in HIV-1 p24 recognized from a long-term nonprogressor: constraints on immune escape associated with targeting a sequence essential for viral replication. *J. Immunol.* 162:3727–34
11. Kokkotou EG, Sankale JL, Mani I, et al. 2000. In vitro correlates of HIV-2 mediated HIV-1 protection. *Proc. Natl. Acad. Sci. USA* 97:6797–802

11a. Gandhi RT, Walker BD. 2002. Immunologic control of HIV-1. *Annu. Rev. Med.* 53:149–72

12. Mellors JW, Rinaldo CR Jr, Gupta P, et al. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272:1167–70
13. Ada GL. 1990. Modern vaccines: the immunological principles of vaccination. *Lancet* 335:523–26
14. Walker CM, Moody DJ, Stites DP, Levy JA. 1986. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* 234:1563–66
15. Cocchi F, DeVico AL, Garzino-Demo A, et al. 1995. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 270:1811–15
16. Parren PW, Burton DR. 2001. The antiviral activity of antibodies in vitro and in vivo. *Adv. Immunol.* 77:195–262
17. Trkola A, Ketas T, Kewalramani VN, et al. 1998. Neutralization sensitivity of human immunodeficiency virus type 1 primary isolates to antibodies and CD4-based reagents is independent of coreceptor usage. *J. Virol.* 72:1876–85
18. Cecilia D, KewalRamani VN, O'Leary J, et al. 1998. Neutralization profiles of primary human immunodeficiency virus type 1 isolates in the context of coreceptor usage. *J. Virol.* 72:698896
19. Siliciano RF, Lawton T, Knall C, Karr RW, Berman P, et al. 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for CD4+ cell depletion. *Cell* 54:561–75

19a. Johnson WE, Desrosiers RC. 2002. Viral persistence: HIV's strategies of immune system evasion. *Annu. Rev. Med.* 53:499–518

20. Prince AM, Reesink H, Pascual D, et al. 1991. Prevention of HIV infection by passive immunization with HIV immunoglobulin. *AIDS Res. Hum. Retroviruses* 7:971–73

20a. Emini EA, Schleif WA, Nunberg JH, et al. 1992. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. *Nature* 355:728–30

21. Putkonen P, Thorstensson R, Ghavamzadeh L, et al. 1991. Prevention of HIV-2 and SIV_{sm} infection by passive immunization in cynomolgus monkeys. *Nature* 352:436–38
22. Shibata R, Igarashi T, Haigwood N, et al. 1999. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat. Med.* 5:204–10

23. Mascola JR, Lewis MG, Stiegler G, et al. 1999. Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J. Virol.* 73:4009–18
24. Baba TW, Liska V, Hofmann-Lehmann R, et al. 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat. Med.* 6:200–6
25. Stott EJ. 1991. Anti-cell antibody in macaques. *Nature* 353:393
26. Stott EJ, Chan WL, Mills KH, et al. 1990. Preliminary report: protection of cynomolgus macaques against simian immunodeficiency virus by fixed infected-cell vaccine. *Lancet* 336:1538–41
27. Langlois AJ, Weinhold KJ, Matthews TJ, et al. 1992. Detection of anti-human cell antibodies in sera from macaques immunized with whole inactivated virus. *AIDS Res. Hum. Retroviruses* 8:1641–52
28. Montefiori DC, Cornell RJ, Zhou JY, et al. 1994. Complement control proteins, CD46, CD55, and CD59, as common surface constituents of human and simian immunodeficiency viruses and possible targets for vaccine protection. *Virology* 205:82–92
29. Haigwood NL, Watson A, Sutton WF, et al. 1996. Passive immune globulin therapy in the SIV/macaque model: early intervention can alter disease profile. *Immunol. Lett.* 51:107–14
30. Koup RA, Safrit JT, Cao YZ, et al. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J. Virol.* 68:4650–55
31. Borrow P, Lewicki H, Hahn BH, et al. 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J. Virol.* 68:6103–10
32. McMichael AJ, Phillips RE. 1997. Escape of human immunodeficiency virus from immune control. *Annu. Rev. Immunol.* 15:271–96
33. Clerici M, Levin JM, Kessler HA, et al. 1994. HIV-specific T-helper activity in seronegative health care workers exposed to contaminated blood. *JAMA* 271:42–46
34. Pinto LA, Sullivan J, Berzofsky JA, et al. 1995. ENV-specific cytotoxic T lymphocyte responses in HIV seronegative health care workers occupationally exposed to HIV-contaminated body fluids. *J. Clin. Invest.* 96:867–76
35. Deacon NJ, Tsykin A, Soloman A, et al. 1995. Genomic structure of an attenuated quasi species of HIV-1 from blood transfusion donor recipients. *Science* 270:988–91
36. Kirchhoff F, Greenough TC, Brettler DB, et al. 1995. Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. *N. Engl. J. Med.* 332:228–32

36a. Pilgrim AK, Pantaleo G, Cohen OJ, et al. 1997. Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term-nonprogressive infection. *J. Infect. Dis.* 176:924–32

37. Rosenberg ES, Billingsley JM, Caliendo AM, et al. 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 278:1447–50
38. Ortiz GM, Nixon DF, Trkola A, et al. 1999. HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy. *J. Clin. Invest.* 104:R13–18
39. Rosenberg ES, Altfeld M, Poon SH, et al. 2000. Immune control of HIV-1 after early treatment of acute infection. *Nature* 407:523–26
40. Reimann KA, Tenner Racz K, Racz P, et al. 1994. Immunopathogenic events in acute infection of rhesus monkeys

with simian immunodeficiency virus of macaques. *J. Virol.* 68:2362–70
41. Hirsch VM, Fuerst TR, Sutter G, et al. 1996. Patterns of viral replication correlate with outcome in simian immunodeficiency virus (SIV)-infected macaques: effect of prior immunization with a trivalent SIV vaccine in modified vaccinia virus Ankara. *J. Virol.* 70:3741–52
42. Ourmanov I, Brown CR, Moss B, et al. 2000. Comparative efficacy of recombinant modified vaccinia virus Ankara expressing simian immunodeficiency virus (SIV) Gag-Pol and/or Env in macaques challenged with pathogenic SIV. *J. Virol.* 74:2740–51
43. Leno M, Kowalski M, Robert-Guroff M. 2000. CD8 T cell anti-HIV activity as a complementary protective mechanism in vaccinated chimpanzees. *AIDS* 14:893–94
44. Allen TM, Vogel TU, Fuller DH, et al. 2000. Introduction of AIDS virus-specific CTL activity in fresh, unstimulated peripheral blood lymphocytes from rhesus macaques vaccinated with a DNA prime/modified vaccinia virus Ankara boost regimen. *J. Immunol.* 164:4968–78
45. Buge SL, Richardson E, Alipanah S, et al. 1997. An adenovirus-simian immunodeficiency virus env vaccine elicits humoral, cellular, and mucosal immune responses in rhesus macaques and decreases viral burden following vaginal challenge. *J. Virol.* 71:8531–41
46. Abimiku AG, Robert-Guroff M, Benson J, et al. 1997. Long-term survival of SIVmac251-infected macaques previously immunized with NYVAC-SIV vaccines. *J. Acq. Immun. Defic. Syndr.* 15: S78–85
47. Davis NL, Caley IJ, Brown KW, et al. 2000. Vaccination of macaques against pathogenic simian immunodeficiency virus with Venezuelan equine encephalitis virus replicon particles. *J. Virol.* 74: 371–78
48. Egan MA, Charini WA, Kuroda MJ, et al. 2000. Simian immunodeficiency virus (SIV) gag DNA-vaccinated rhesus monkeys develop secondary cytotoxic T-lymphocyte responses and control viral replication after pathogenic SIV infection. *J. Virol.* 74:7485–95
49. Barouch DH, Santra S, Schmitz JE, et al. 2001. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. *Science* 290:486–92
50. Amara RR, Villinger F, Altman JD, et al. 2001. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 292:69–74
51. Cafaro A, Caputo A, Fracasso C, et al. 1999. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nat. Med.* 5:643–50
52. Graham BS, Karzon DT. 1998. AIDS vaccine development. In *Textbook of AIDS Medicine*, ed. TC Merigan, JG Bartlett, D Bolognesi, 42:689–724. Baltimore, MD: Williams & Wilkins. 1063 pp. 2nd ed.
53. Graham BS. 2000. Clinical trials of HIV vaccines. In *HIV Molecular Immunology 2000*, ed. BTM Korber, JP Moore, C Brander, et al., p. I-20–I-38. Los Alamos, NM: Los Alamos Natl. Lab., Theor. Biol. Biophys. http://hiv-web.lanl.gov/immunology
54. Graham BS. 2001. NIH Vaccine Research Center Clinical Studies. http://www.vrc.nih.gov/VRC/clinstudies.htm
55. Keefer MC, Wolff M, Gorse GG, et al. 1997. Safety profile of phase I and II preventive HIV-1 vaccination: experience of the AIDS Vaccine Evaluation Group. *AIDS Res. Hum. Retroviruses* 13:1163–77
56. Graham BS, Matthews TJ, Belshe RB, et al. 1993. Augmentation of human immunodeficiency virus type 1 neutralizing antibody by priming with gp160 recombinant vaccinia and boosting with rgp160 in vaccinia-naive adults. *J. Infect. Dis.* 167:533–37
57. Berman PW, Huang W, Riddle L, et al.

1999. Development of bivalent (B/E) vaccines able to neutralize CCR5-dependent viruses from the United States and Thailand. *Virology* 265:1–9

58. Cooney EL, McElrath MJ, Corey L, et al. 1993. Enhanced immunity to human immunodeficiency virus (HIV) envelope elicited by a combined vaccine regimen consisting of priming with a vaccinia recombinant expressing HIV envelope and boosting with gp160 protein. *Proc. Natl. Acad. Sci. USA* 90:1882–86
59. Corey L, McElrath MJ, Weinhold K, et al. 1998. Cytotoxic T cell and neutralizing antibody responses to HIV-1 envelope with a combination vaccine regimen. *J. Infect. Dis.* 177:301–9
60. Hammond SA, Bollinger RC, Stanhope PE, et al. 1992. Comparative clonal analysis of human immunodeficiency virus type 1 (HIV-1)-specific CD4+ and CD8+ cytolytic T lymphocytes isolated from seronegative humans immunized with candidate HIV-1 vaccines. *J. Exp. Med.* 176:1531–42
61. El-Daher N, Keefer MC, Reichman RC, et al. 1993. Persisting human immunodeficiency virus type 1 gp160-specific human T lymphocyte responses including CD8+ cytotoxic activity after receipt of envelope vaccines. *J. Infect. Dis.* 168:306–13
62. Clements-Mann ML, Matthews TJ, Weinhold K, et al. 1998. HIV-1 immune responses induced by canarypox (ALVAC)-gp160 MN, SF-2 rgp120, or both vaccines in seronegative adults. *J. Infect. Dis.* 177:1230–46
63. Evans TG, Keefer MC, Weinhold KJ, et al. 1999. A canarypox vaccine expressing multiple HIV-1 genes given alone or with rgp120 elicits broad and durable CD8+ CTL responses in seronegative volunteers. *J. Infect. Dis.* 180:290–98
64. Belshe RB, Gorse GJ, Mulligan MJ, et al. 1998. Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. *AIDS* 12:2407–15
65. Salmon-Ceron D, Excler JL, Finkielsztejn L, et al. 1999. Safety and immunogenicity of a live recombinant canarypox virus expressing HIV type 1 gp120 MN tm/gag/protease LAI (ALVAC-HIV, vCP205) followed by a p24E-V3 MN synthetic peptide (CLTB-36) administered in healthy volunteers at low risk for HIV infection. *AIDS Res. Hum. Retroviruses* 15:633–45
66. Belshe RB, Stevens C, Gorse GJ, et al. 2001. Safety and immunogenicity of a canarypox-vectored human immunodeficiency virus type 1 vaccine with or without gp120: a phase 2 study in higher- and lower-risk volunteers. *J. Infect. Dis.* 183:1343–52
67. Ferrari G, Humphrey W, McElrath MJ, et al. 1997. Clade B–based HIV-1 vaccines elicit cross-clade cytotoxic T lymphocyte reactivities in uninfected volunteers. *Proc. Natl. Acad. Sci. USA* 94:1396–401
68. Castillo RC, Arango-Jaramillo S, John R, et al. 2000. Resistance to human immunodeficiency virus type 1 in vitro as a surrogate of vaccine-induced protective immunity. *J. Infect. Dis.* 181:897–903
69. Murali-Krishna K, Altman JD, Suresh M, et al. 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 8:177–87